

CLAIMS

1. Isolated DNA which is the *ced-3* gene.
2. Isolated DNA having the nucleotide sequence of Figure 4 (Seq. ID #18) A
- 5 3. Isolated DNA encoding the amino acid sequence of Figure 4 (Seq. ID #19).
4. Isolated RNA encoded by the DNA of Claim 1.
- Ins. A2 5. Isolated protein encoded by the DNA of Claim 1.
- 10 6. Isolated protein having the amino acid sequence of Figure 4 (Seq. ID #19).
7. An antibody directed against the protein of Claim 6.
- 15 8. Isolated DNA which is a mutated *ced-3* or *ced-4* gene having a mutation which affects the activity of the gene. A
9. The DNA of Claim 8, wherein the mutated *ced-4* gene is selected from the group consisting of:
- 20 a) n1162;
- b) n2274;
- c) n1920;
- d) n2247;
- e) n2273;
- f) n1948;
- 25 g) n1947; and
- h) n1894.

10. The DNA of Claim 8, wherein the mutation in *ced-4* results in an alteration selected from the group consisting of:

- a) Q to termination at codon 40;
- b) R to termination at codon 139;
- c) I to N at codon 258;
- d) Q to termination at codon 262;
- e) W to termination at codon 401; and
- f) an alteration in mRNA splicing resulting from a change at nucleotide 6297.

11. The DNA of Claim 8, wherein the mutation in *ced-4* is selected from the group consisting of:

- a) C to T at nucleotide 1131;
- b) C to T at nucleotide 1428;
- c) G to A at nucleotide 1929;
- d) T to A at nucleotide 2117;
- e) C to T at nucleotide 2128; and
- f) G to A at nucleotide 3131.

12. The DNA of Claim 8, wherein the mutated *ced-3* gene is selected from the group consisting of:

- a) n1040;
- b) n718;
- c) n2433;
- d) n1164;
- e) n717;
- f) n1949;
- g) n1286;
- h) n1129;
- i) n1165;
- j) n2430;
- k) n2426; and
- l) n1163.

13. The DNA of Claim 8, wherein the mutation in *ced-3* results in an alteration selected from the group consisting of:

- a) L to F at codon 27;
- 5 b) G to R at codon 65;
- c) G to S at codon 360;
- d) Q to termination at codon 403;
- e) Q to termination at codon 417;
- f) W to termination at codon 428;
- 10 g) A to V at codon 449;
- h) A to V at codon 466;
- i) E to K at codon 483;
- j) S to F at codon 486; and
- 15 k) an alteration in mRNA splicing at nucleotide 6297.


14. The DNA of Claim 8, wherein the mutation in *ced-3* is selected from the group consisting of:

- a) C to T at nucleotide 2310;
- b) G to A at nucleotide 2487;
- 20 c) G to A at nucleotide 5757;
- d) C to T at nucleotide 5940;
- e) G to A at nucleotide 6297;
- f) C to T at nucleotide 6322;
- g) G to A at nucleotide 6342;
- 25 h) C to T at nucleotide 6434;
- i) C to T at nucleotide 6485;
- j) G to A at nucleotide 6535; and
- k) C to T at nucleotide 7020.

15. Isolated RNA encoded by the DNA of Claim 8.

16. Isolated protein encoded by the DNA of Claim 8.

Ins:  30

- 004260 16372660
17. Isolated DNA which is a gene selected from the group consisting of:
- a) a gene which is structurally related to the *ced-3* gene;
 - 5 b) a gene which is functionally related to the *ced-3* gene;
 - c) a gene which is both structurally and functionally related to the *ced-3* gene;
 - d) a gene which is structurally related to the *ced-4* gene;
 - 10 e) a gene which is functionally related to the *ced-4* gene; and
 - f) a gene which is both structurally and functionally related to the *ced-4* gene.
- 15 18. Isolated RNA encoded by the DNA of Claim 17.
-  19. Isolated protein encoded by the DNA of Claim 17.
20. An antibody directed against the protein of Claim 19.
21. A probe for identifying a gene which is structurally related to the *ced-3* gene, said probe which is selected from the group consisting of:
- a) DNA having all or a portion of the nucleotide sequence of Figure 4 (Seq. ID #18);
 - b) RNA encoded by the DNA of a);
 - 25 c) degenerate oligonucleotides derived from a portion of the amino acid sequence of Figure 4 (Seq. ID #19); and
 - d) an antibody directed against the protein of c).

22. A probe for identifying a gene which belongs to the same gene family as the *ced-3* gene, said probe which is selected from the group consisting of:
- 5 a) all or a portion of a gene which is structurally related to *ced-3*;
 - b) RNA encoded by a);
 - c) DNA having the consensus sequence of a conserved region between at least two other genes which belong to said gene family;
 - 10 d) RNA encoded by c);
 - e) degenerate oligonucleotides derived from a portion of the amino acid sequence of a protein encoded by a);
 - 15 f) degenerate oligonucleotides derived from the consensus sequence of a conserved region between the proteins encoded by at least two other genes which belong to said gene family; and
 - 20 g) an antibody directed against all or a portion of a protein encoded by a).
23. A method for identifying a gene which is structurally related to a cell death gene selected from *ced-3* and *ced-4*, comprising the steps of:
- 25 a) combining DNA with a nucleic acid probe comprising said cell death gene, or a portion able to specifically hybridize to said cell death gene, under conditions suitable for specific hybridization of the nucleic acid probe to complementary sequences; and
 - 30 b) detecting specific hybridization of the nucleic acid probe to the DNA, wherein specific hybridization indicates that a structurally related gene, or portion, is present in the DNA,

thereby identifying a gene which is structurally related to a cell death gene selected from *ced-3* and *ced-4*.

- 5 24. The method of Claim 23, wherein the DNA is a gene library.
25. The method of Claim 23, wherein the nucleic acid probe further comprises degenerate oligonucleotides derived from the amino acid sequence of the product of the cell death gene.
- 10 26. A method for identifying a gene which is structurally related to a cell death gene selected from *ced-3* and *ced-4*, comprising the steps of:
- 15 1) combining nucleic acid with primers comprising portions of said cell death gene under conditions suitable for polymerase chain reaction; and
- 20 2) detecting specific DNA amplification, wherein specific DNA amplification produces a structurally related gene, or portion, thereby identifying a gene which is structurally related to a cell death gene selected from *ced-3* and *ced-4*.
- 25 27. The method of Claim 26, wherein the primers further comprise degenerate oligonucleotides derived from the amino acid sequence of the product of the cell death gene.

28. A method for identifying a gene which is structurally related to a cell death gene selected from *ced-3* and *ced-4*, comprising the steps of:
- a) combining an expression gene library with an antibody directed against the protein encoded by said cell death gene under conditions suitable for specific antibody-antigen binding of the antibody to antigens expressed from the gene library; and
 - b) detecting specific antibody-antigen binding, wherein specific antibody-antigen binding indicates that a structurally related gene is present in the expression gene library, thereby identifying a gene which is structurally related to a cell death gene selected from *ced-3* and *ced-4*.
29. A bioassay for identifying a cell death gene, comprising the steps of:
- a) using a gene and a nematode selected from a nematode having reduced activity of a cell death gene and a wild-type nematode to produce a transgenic nematode; and
 - b) determining in said transgenic nematode an increase in cell deaths which occur during the development of the nontransgenic nematode, wherein an increase in cell deaths indicates the activity of a cell death gene, thereby identifying a cell death gene.
30. The bioassay of Claim 29, wherein the nematode underexpresses or expresses an inactivated form of a gene selected from *ced-3* and *ced-4*.

31. The bioassay of Claim 29, wherein the gene is from an organism other than a nematode.
32. The bioassay of Claim 29, wherein the gene is a component of an expression gene library.
- 5 33. Isolated DNA which is a cell death gene identified by the bioassay of Claim 29.
34. A bioassay to identify a mutation in a cell death gene which alters the activity of the gene, comprising the steps of:
- 10 a) using a mutated cell death gene and a nematode selected from a nematode having reduced activity of a cell death gene and a wild-type nematode to produce a transgenic nematode; and
- 15 b) comparing cell deaths which occur during the development of the transgenic nematode having the mutated gene with those which occur in a transgenic nematode having a non-mutated gene, wherein a difference in cell deaths
- 20 indicates that the mutation alters the activity of the cell death gene, thereby identifying a mutation in a cell death gene which alters the activity of the gene.
35. Isolated DNA which is a cell death gene having a
- 25 mutation identified by the bioassay of Claim 34.
36. The isolated DNA of Claim 35, wherein the mutation has a result selected from the group consisting of:
- a) inactivation of the cell death gene;

- b) constitutive activation of the cell death gene; and
- c) production of a mutated gene which does not cause cell death and which antagonizes the activity of functioning cell death genes.
- 5
37. A bioassay for identifying a gene which affects the activity of a cell death gene, comprising the steps of:
- a) using a gene and a nematode containing a cell death gene to produce a transgenic nematode; and
- 10
- b) determining in said transgenic nematode a difference in cell deaths from cell deaths which occur during the development of the nontransgenic nematode, wherein a difference in cell deaths indicates a gene which affects the activity of a cell death gene,
- 15
- thereby identifying a gene which affects the activity of a cell death gene.
- 20 38. The bioassay of Claim 37, wherein the cell death gene is selected from the group consisting of:
- a) a wild-type gene;
- b) an underexpressed gene;
- c) a gene having reduced activity;
- 25
- d) an overexpressed gene; and
- e) a gene having hyperactivity.
39. The bioassay of Claim 37, wherein the gene is a component of an expression gene library.
40. An isolated gene identified by the bioassay of Claim 37.
- 30

41. A bioassay for identifying an agent which mimics the activity of a cell death gene, comprising the steps of:
- a) introducing an agent into a nematode selected from a nematode having reduced activity of a cell death gene and a wild-type nematode; and
 - b) detecting an increase in cell deaths which occur in the nematode, wherein an increase indicates that the agent mimics the activity of a cell death gene,
- thereby identifying an agent which mimics the activity of a cell death gene.
42. The bioassay of Claim 41, wherein the nematode underexpresses or expresses an inactivated gene selected from *ced-3* or *ced-4*.
43. The bioassay of Claim 42, wherein the agent is introduced into the nematode by a method selected from: microinjection, diffusion, ingestion and shooting in with a particle gun.
44. An agent identified by the bioassay of Claim 41.
45. A bioassay for identifying an agent which affects the activity of a cell death gene, comprising the steps of:
- a) introducing an agent into a nematode which expresses a cell death gene; and
 - b) detecting a change in the pattern of cell deaths which occur in the development of the nematode, wherein a change indicates that the agent affects the activity of the cell death gene,

thereby identifying an agent which affects the activity of a cell death gene.

- 5 46. The bioassay of Claim 45, wherein the nematode expresses an endogenous cell death gene or a cell death gene which is a transgene.
47. The bioassay of Claim 46, wherein the cell death gene is *ced-3* or *ced-4*.
- 10 48. The bioassay of Claim 45, wherein the nematode overexpresses or underexpresses the cell death gene.
49. The bioassay of Claim 45, wherein the nematode expresses an inactivated or constitutively activated form of the cell death gene.
- 15 50. The bioassay of Claim 45, wherein the nematode underexpresses or expresses an inactivated form of a gene selected from *ced-3* and *ced-4*.
51. An agent identified by the bioassay of Claim 45.
- 20 52. The agent of Claim 47 which is selected from the group consisting of:
- a) single stranded nucleic acid having all or a portion of the antisense sequence of the cell death gene which is complementary to the mRNA encoded by the gene;
 - b) DNA encoding a); and
 - 25 c) an antagonist of the cell death gene.

53. A method for altering the occurrence of cell death, comprising altering in the cell the activity of a cell death gene.
54. The method of Claim 53, wherein the cell death gene is *ced-3* or *ced-4*.
55. The method of Claim 53, comprising exposing the cell to an agent which alters or mimics the activity of a cell death gene in the cell under conditions appropriate for activity of the agent.
56. The method of Claim 55, wherein the activity of the cell death gene is increased, comprising exposing the cell to an agent selected from the group consisting of:
- a) DNA comprising the cell death gene, or active portion thereof;
 - b) RNA encoded by the cell death gene, or active portion thereof;
 - c) protein encoded by the cell death gene, or active portion thereof;
 - d) an agent which is structurally similar to and mimics the activity of the protein encoded by the cell death gene;
 - e) DNA comprising a constitutively activated form of a cell death gene, or active portion thereof;
 - f) RNA encoded by the DNA of e), or active portion thereof;
 - g) protein encoded by the DNA of e), or active portion thereof;
 - h) an agent which is structurally similar to and mimics the activity of the protein encoded by the DNA of a); and

i) an agonist of the cell death gene,
under conditions appropriate for the activity of
the agent.

57. The method of Claim 55, wherein the activity of
the cell death gene is decreased, comprising
exposing the cell to an agent selected from the
group consisting of:

- a) single stranded nucleic acid having all or a
portion of the antisense sequence of the cell
death gene which is complementary to the mRNA
of the gene;
- b) DNA which directs the expression of a);
- c) a mutated cell death gene which does not
cause cell death and which antagonizes the
activity of the cell death gene;
- d) RNA encoded by c);
- e) protein encoded by c); and
- f) an antagonist of the cell death gene,
under conditions appropriate for the activity of
the agent.

58. A method for reducing the proliferative capacity
or size of a population of cells, comprising
increasing the activity of a cell death gene in
the cells.

59. The method of Claim 58, wherein the cells are
selected from:

- a) cancerous cells;
- b) infected cells;
- c) cells producing autoreactive
antibodies; and
- d) hair follicle cells.

60. The method of Claim 58, wherein the cell death gene is selected from the group consisting of:
- a) *ced-3*;
 - b) a cell death gene which is structurally related to *ced-3*; and
 - c) a gene which is functionally related to *ced-3*.
61. The method of Claim 58, wherein the cell death gene is selected from the group consisting of:
- a) *ced-4*;
 - b) a cell death gene which is structurally related to *ced-4*; and
 - c) a gene which is functionally related to *ced-4*.
62. A method for treating a condition characterized by cell deaths, comprising decreasing the activity of a cell death gene.
63. The method of Claim 62, wherein the condition is selected from the group consisting of:
- a) myocardial infarction;
 - b) stroke;
 - c) degenerative disease;
 - d) traumatic brain injury;
 - e) hypoxia;
 - f) pathogenic infection;
 - g) aging; and
 - h) hair loss.

64. A method for treating a parasitic infection of a host animal, comprising administering an agent which increases the activity of a cell death gene specific to the parasite and which does not harm the host animal.
65. The method of Claim 64, wherein the parasite is a nematode.
66. A method for incapacitating or killing a pest, comprising increasing the activity of a cell death gene in the pest.
67. A method of biological containment of a recombinant organism, comprising introducing in the organism nucleic acid which is able to direct the expression of an agent which increases the activity of a cell death gene in the organism under predetermined conditions, thereby incapacitating or killing the recombinant organism.
68. The method of Claim 67, wherein the agent kills the recombinant organism upon completion of a desired task by the organism.

034359 667550

Sept
04